植物保護學會會刊 40：397～408, 1998

Root Rot of Vegetable Pea Seedlings in Soilless Cultural System Caused by *Pythium aphanidermatum* and *P. ultimum*

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(Accpted for publication: October 6, 1998)

ABSTRACT

Huang, J. H., and Lin, Y. S. 1998. Root rot of vegetable pea seedlings in soilless cultural system caused by *Pythium aphanidermatum* and *P. ultimum*. Plant Prot. Bull. 40: 397-408.

A previously undiagnosed root rot was found to be a limiting factor for commercial production of vegetable pea seedlings in a soilless cultivation system under shade in Taiwan. Infected seedlings either died or were severely stunted. Both *Pythium aphanidermatum* and *P. ultimum* were associated with the disease. Inoculation tests revealed that the infection temperatures for *P. aphanidermatum* and *P. ultimum* were 20-32 and 12-28℃, respectively. When both pathogens were present in the commercial cultivation system or employed in the growth chamber inoculation tests, *P. aphanidermatum* would predominate and became the primary causal pathogen of root rot during the warm summer, with an air temperature higher than 24℃; however, *P. ultimum* played a more important role in causing root rot during the cool winter, when air temperature was lower than 20℃.

(Key words: vegetable pea seedlings, *Pisum sativum*, root rot, *Pythium aphanidermatum, P. ultimum*)

INTRODUCTION

Pea (*Pisum sativum* L.) seedlings (also named pea sprouts by consumers improperly) have been commercially produced as vegetable in recent years in Taiwan [3, 4]. A previously undiagnosed root rot was observed for the first time in summer 1990 at Lung-Tein area, southern part of Taiwan. *Pythium aphanidermatum* (Edson) Fitzp. was consistently isolated from rotted root tissues of decaying seedlings. The air temperature under the shade at this time was 26-28 ℃ or so. It was thought that *P. aphanidermatum*, a known pathogen causing root disease at high temperature, would not be a problem in continuing cultivation during cool winter. However, stunting and death of pea seedlings continued to occure in December-February eventhough the air temperatures declined to less than 20℃. Results of isolation tests on diseased plants carried out in winter time indicated that *P. ultimum* Traw became the predominated species in association with diseased plants. This disease occurred in all commercial pea seedling farms in Taiwan and many of them were subsequently abandoned.

The incidence of disease, pathogenicity of the two *Pythium* species, *P. aphanidermatum* and *P. ultimum*, and their seasonal occurrence as affected by temperature in naturally infested commercial farms are presented in this paper.

MATERIALS AND METHODS

Cultivation of vegetable pea seedlings

The cultivation procedures for vegetable pea seedlings used by farmers are employed in this study. About 1,200 g of 'Dun peas' or 'Dundale pea' seeds imported from Australia were soaked in water for 12-18 hr, and then evenly distributed into one layer over the cultural substrates (saw dust) in a seedling tray (60×30×3 cm) for growing seedlings. The trays with pea seeds were incubated at high humidity in dark for 4 days for seed germination and seedling growth, then they were moved to a plastic sheet house, which had 40-50% of sunlight cut off. Greening of seedlings took place after 4 days of incubation in summer and 8 days or more in winter. To produce tender and succulent shoots, the seedlings were irrigated with tap water 2-4 times a day to maintain a higher moist environment. When the seedlings had grown up to 12 cm, the shoots were harvested by cutting at the basal stems [4]. After seedlings being harvested, the used cultural trays were refilled with new cultural substrate for next planting.

Disease survey and isolation of pathogens

Disease survey were made in vegetable pea seedling farms in Taiwan in the summer or winter of 1990-1992. The diseased pea seedlings with root rot symptoms were collected and washed in running tap water, cut into pieces (1 cm long), rinsed in sterile water for three times, blotted dry with paper towel, and then placed on 2% water agar. After 48 hr incubation at 24-26℃, hyphal tips of fungi growing out from the root segments were cut and transferred onto 2% water agar, potato carrot agar and V-8 agar for identification [11].

To determine the pathogen occurring in different seasons, extensive attempts to isolate the pathogens were made twice in February (winter) and July (summer) of 1992 from Wu-Feng farm. One hundred pea seedlings were randomly collected daily from 25 cultural trays throughout the planting period, in other words, within 15 days after seeding. Harvest of the seedlings was done at the 9th day of winter

planting and at 7th day of summer planting and no more irrigation was given to the cultural trays after harvest. The collected samples were subjected to isolation tests using 2% water agar in laboratory.

Pathogenicity test

Fungi isolated from diseased specimens included *P. aphanidermatum* (P324), *P. ultimum* (P322), *Mucor* spp., *Fusarium* spp. and *Dactylaria* spp.. They were subjected to the pathogenicity tests conducted in October 1991 in greenhouse. Mycelial discs of a 72 hr-old V-8 agar culture plate of the test fungi were put in a 250 ml flask containing 0.5 g of oat powder and 100 ml of potato dextrose broth, and incubated at 24℃ for 14 days. The mycelial mats were removed, rinsed twice with distilled water, homogenized in 50 ml distilled water in Warning blender (National Co., Taiwan), and then mixed with 100 g of saw dust as inoculum. Then, 100 g of the inoculum were mixed with 500 g of non-infested saw dust as cultural substrate and put on new cultural trays for growing pea seedlings in the greenhouse. Noninfested substrate served as controls. There were 5 replications in each treatment and the experiment was repeated once. Symptom severity was recorded 8 days after seeds being inoculated by a scouting scale of 0-3, where 0=over 12 cm; 1=8.1-12 cm; 2=4.1-8.0 cm; and 3=less than 4.0 cm of plant height. The ratings are then converted into disease severity (P) by the following formula:

$$P\ (\%) = \frac{\sum n_i \times i}{N \times 3} \times 100\ \%$$

Where n_i is the number of plants in each symptom category of i and N is the total number of plants rated.

Twenty seedlings were sampled from each treatment and placed on 2% water agar for reisolation of the pathogens.

Disease incidence and seasonal occurrence of pathogens

A new operated commercial farm for culturing pea seedling at Wu-Feng area in the central part of Taiwan was selected for conducting the following experiments. Disease surveys were made biweekly during the period of July 1991 to June 1993. For each disease survey, 30 cultural trays were picked up randomly and the percentage of area with diseased seedlings in each sampled trays were determined as disease incidence. Fifty root systems were also sampled randomly from the 30 trays for isolation of the pathogens on 2% water agar. After sampling, the remaining seedlings in each tray were harvested by cutting at the basal part of the stems. Shoot weights were measured and recorded. The average weight of pea shoots from the 30 trays was served as the yield per tray. Data logger (Li-1000, Licor, Co., U. S. A.) was set up in the farm to record the daily air humidities, substrate temperature and air temperature 30 cm above the plant canopy.

Temperature requirement

To determine the optimum temperature for mycelial growth, *P. aphanidermatum and P. ultimum* were grown on 2%potato-carrot agar [13] for 3 days at 24℃. The mycelial discs (0.8 cm) were cut with a cork borer from the periphery of the colonies, then transferred onto the edge of a plate with 2% potato carrot agar and incubated in the 4 - 44℃ incubators with 4℃ intervals. After 12-hr incubation, the colony radius was started to record as the daily growth rate of mycelium.

To determine the temperature effects on the

root rot of pea seedlings, the saw dust substrate infested with *P. aphanidermatum* or *P. ultimum* alone or both was employed to grow pea seedlings on new cultural trays in plant growth chambers (Yo Tein Co., Taiwan) at 12, 16, 20, 24, 28 and 32℃. Noninfested substrate served as controls. All treatments had 5 replications and the experiment was repeated once. Disease severity was evaluated at 8, 11 and 17 days after seeding.

Experiments were also conducted to determine the effect of temperature on the infection of roots by *P. aphanidermatum* and *P. ultimum*. Pea seeds were sowed on the substrate previously infested with one pathogen, *P. aphanidermatum* or *P. ultimum*, and incubated in the plant growth chambers at 16, 20, 24 and 28℃ for 3 days. Then, the infected pea seedlings were transplanted into the substrate infested with the other pathogen, *P. ultimum* or *P. aphanidermatum*, and incubated at the same temperature for another 7 days. Substrates infested singly with either *P. aphanidermatum* or *P. ultimum* served as controls. All treatments had 5 replications and the experiment was repeated once. Pathogen isolations were made on 2% water agar and recorded at 3 and 10 days after seeding.

RESULTS

Symptoms, pathogens isolation and pathogenicity tests

Symptoms of infected seedlings consisted of black seed coat, extensive root rotting, severe stunting, and damping-off, followed shortly by plant death (Fig. 1). The disease caused 50-90% yield reduction in summer and 20-50% in winter during the survey period of 1990 to 1992. Although several fungi, including *P. aphanidermatum* in summer, *P. ultimum* in winter (Table 1) and a few other species such as *Mucor* spp. *Fusarium* spp. and *Dactylaria* spp. were isolated from pea seedlings at different ages (up to 15 days old after seeding) (Table 2), only *P. aphanidermatum* and *P. ultimum* were able to produce symptoms in the greenhouse inoculation tests (Table 3) that identical to those observed in the naturally infested pea seedling farms.

Pathogens identification

The isolate P322 formed 1 or 2 antheridia attached monoclinously or sometimes hypogynously to smooth-walled oogonia (20.4-25.5, av. 22.9 μm). The oospores were usually aplerotic, and 16.3-22.8 (av. 19.4)μm in diameter. No zoospores produced with the methods of Plaats-Niterink [11]. Hyphae swellings (20.4-28.5, av. 23.5μm) were mostly globose and intercalary or terminal. The morphology of this fungus was identical as *Pythium ultimum* var. *ultimum*. The isolate P324, which grew well at 36℃, produced lobate sporangia as swollen hyphal branches. The oogonia had mostly 1 or 2 monoclinous or diclinous antheridia. They were usually terminal, smooth and 22.7-29.7 (av. 26.4) μm in diameter. The oospores were aplerotic and 20.1-24.3 (av. 22.4) μm in diameter. This fungus was identified as *Pythium aphanidermatum* [11].

Table 1. Isolation rates of Pythium spp. from diseased pea seedlings at various farms[1)]

Farm location	Isolation rate (%)[2)]			
	Summer		Winter	
	P.a.[3)]	P.u.	P.a.	P.u.
Lung-Tein	90	0	-[4)]	-
Wu-Feng	94	0	2	16
Hsing-Sheh	90	0	25	40
Ping-Tung	36	0	0	16

1) The data were collected during 1990 to 1992.
2) In each isolation attempt, roots from 100 diseased plants were used.
3) P.a.=*Pythium aphanidermatum*; P.u.=*P. ultimum*.
4) Not tested.

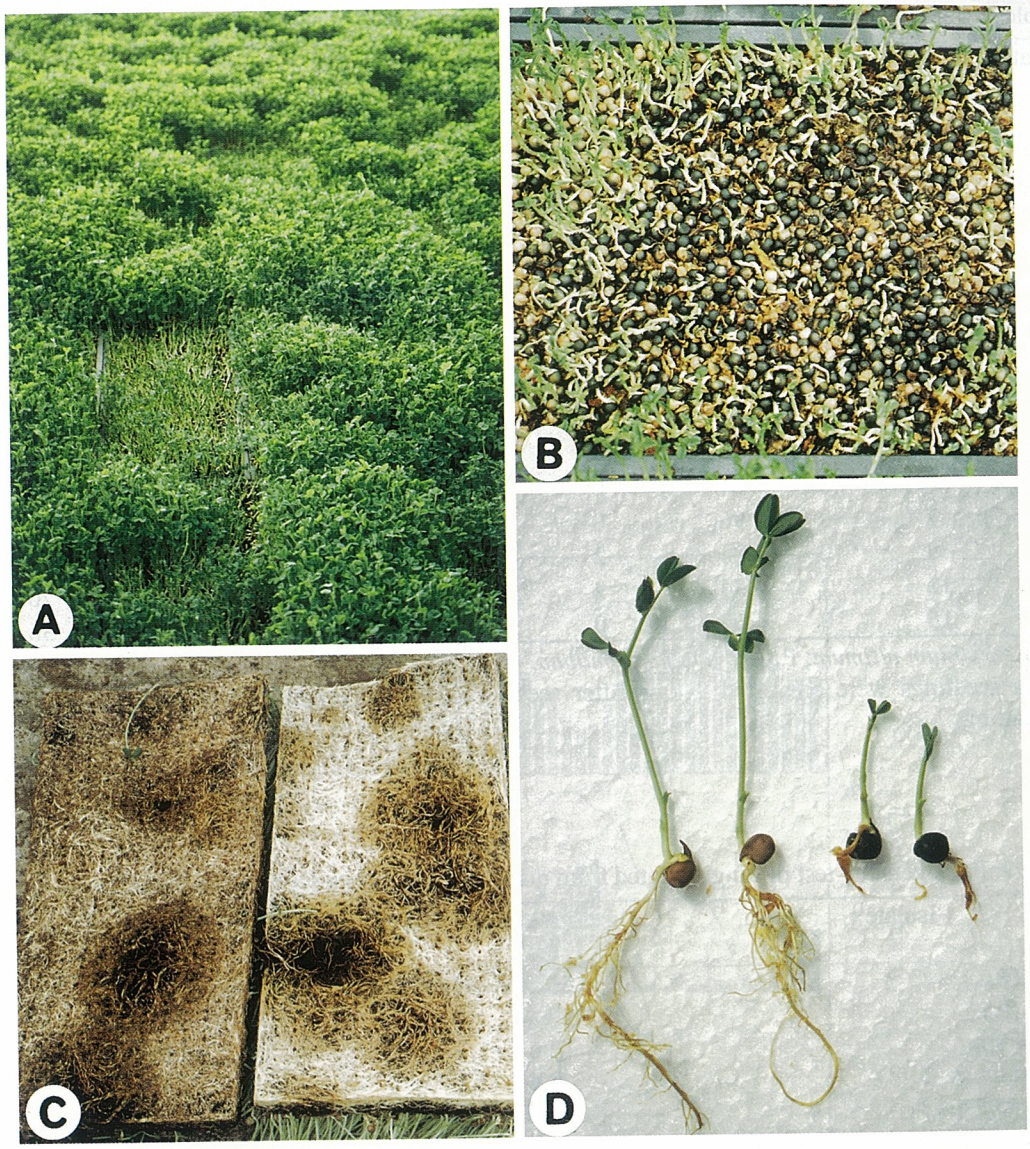

Fig 1. Symptoms of *Pythium* root rot of vegetable pea seedlings: Stunt (A), death (B) and root rot (D) of seedlings. Black color of bottom side of seedling mat from a cultural tray (C).

Disease incidence and seasonal occurrence of pathogens

At Wu-Feng farm, average humidities of one planting period over survey time were often above 80%RH, and did not change significantly. Mean substrate temperatures and the air temperatures 30 cm above the plant canopy were not much different during the test period. Incidence of root rot of pea seedlings caused by *Pythium* spp. occurred continously over the 2-year test period, and was found to relate to the temperature. The disease incidence was high (up to 70%) in summer season when the average temperatures were above 24℃. Over 90% of the

Table 2. Fungi isolated from diseased roots of pea seedlings sampled at different times after seeding

Date	Days after seeding	Isolation rate (%)					
		P.u.[1]	P.a.	*Mucor* spp.	*Fusarium* spp.	*Dactylaria* spp.	Others
1992	2	0	0	0	0	0	0
Feb.	3	0	0	0	0	0	0
	4	12	0	0	0	0	0
	6	30	0	0	0	0	0
	9 [2]	52	0	0	6	0	0
	12	46	6	0	12	4	0
	15	52	4	0	54	34	16
July	2	0	0	4	0	0	0
	3	0	6	2	2	0	0
	4	0	18	0	2	0	8
	5	0	62	0	0	0	2
	6	0	84	6	2	0	0
	7 [2]	0	96	0	2	24	2
	9	0	84	2	14	72	24
	12	2	54	2	44	82	20
	15	0	60	0	70	34	36

[1] P.u.=*Pythium ultimum*; P.a.=*P. aphanidermatum*.

[2] Pea seedlings were harvested 9 days after seeding in Feburary and 7 days after seeding in July plantings.

Table 3. Pathogenicity test of fungi isolated from diseased roots of pea seedlings in greenhouse

Isolate[1]	Disease severity[2] (%)	Reisolation rate (%)
None	3 c[3]	0
P322 (P.u.)	86 b	100
P324 (P.a.)	99 a	90
Mucor spp.	2 c	20
Fusarium sp.-1	3 c	35
Fusarium sp.-2	4 c	10
Dactylaria spp.	1 c	10

[1] P.a.=*Pythium aphanidermatum*; P.u.=*P. ultimum*.

[2] Disease severity was recorded 10 days after inoculation on a scale of 0-3, where 0=Over 12 cm; 1=8.1-12 cm; 2=4.1-8.0 cm;and 3=Less than 4.0 cm of plant height. The ratings are converted to disease severity (P) by the following formula:

$$P\ (\%) = \frac{\sum n_i \times i}{N \times 3} \times 100\ \%$$

Where n_i is the number of plants in each symptom category of i and N is the total number of plants rated.

[3] Values followed by the same letter in each column are not significantly different at p=0.05, according to the Duncan's Multiple Range Test.

pea seedlings were infected by *P. aphanidermatum*, and the yields were lower than 500 g/tray, with 197 g/tray being the lowest. When the average temperatures decreased from 24 to 20°C, disease incidence was reduced and the yield increased sharply. But *P. aphanidermatum* still could be isolated from 54-84% of pea seedlings. Coincided with a drop of temperature approximately from 20 to 14°C in January and February, frequencies of *P. aphanidermatum* isolation quickly falled to 2% and the yields could be up to 783 g/tray. By contrast, when the average temperature increased from 16 to 20°C in March and April, *P. ultimum* became the predominant pathogen with isolation frequency over 80%, and it caused another peak of severe disease. The isolation frequencies of *P. ultimum* decreased when the temperature increased from 20 to 24 °C , but then *P. aphanidermatum* appeared to be the predominant pathogen causing another peak of severe disease (Fig. 2).

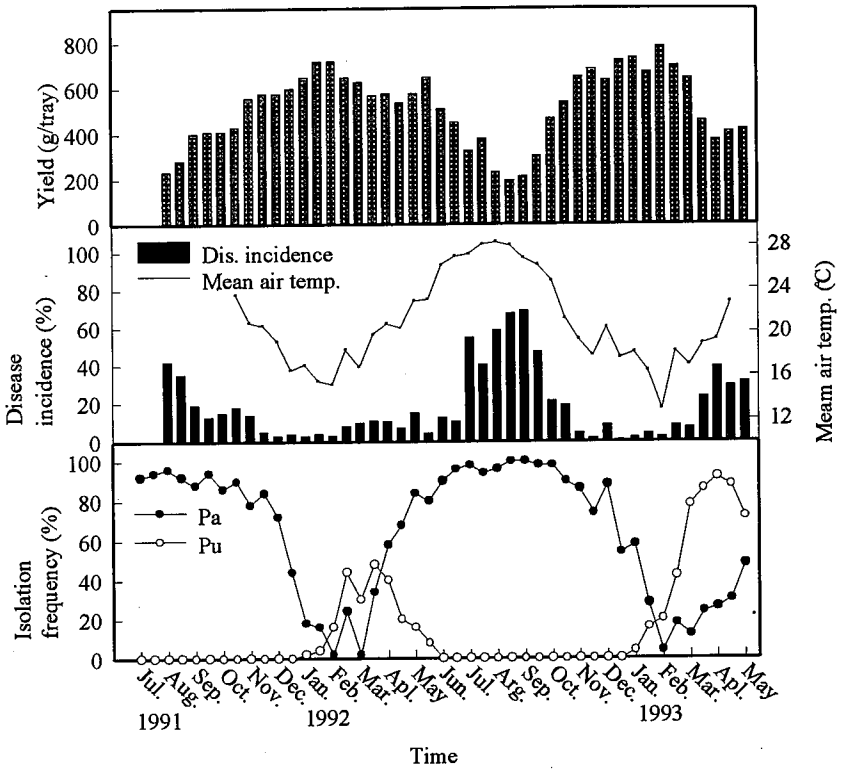

Fig. 2. The fluctuation of root rot of pea seedlings caused by *Pythium aphanidermatum* (P.a.) and *P. ultimum* (P.u.) at Wu-Feng farm. For each survey, seedlings in 30 cultural trays were scouted for the percentage of area with diseased seedlings in each tray as disease incidence. Fifty root systems were also collected randomly from the 30 trays and plated on 2% water agar for isolation of the pathogens. The average weight of pea shoots from the 30 trays was measured. Data logger was set up to record the air humidity and air temperature at 30 cm above the plant canopy.

Temperature requirement

The optimum temperatures for mycelial growth of *P. aphanidermatum* and *P. ultimum* were 36 and 28℃, respectively. The mycelial growth of *P. ultimum* was evidently faster than *P. aphanidermatum* at the temperature below 20℃ (Fig. 3).

The inoculation tests in plant growth chamber showed that pea seedlings grew best at 20 and 24℃, and could be harvested at the 8th day after seeding when pea seedlings reached 9 cm in height. The seedlings grew slower at 16 and 12℃, and could be harvested 11 and 17 days after seeding, respectively. The seedling growth was poor at 28 and 32℃ and the seedlings could not reach the height suitable for market purposes.

Both *P. aphanidermatum* and *P. ultimum* were pathogenic to pea seedlings and caused significant stunt at 20-32 and 12-28 ℃, respectively. In cases that temperature above 24 ℃, *P. aphanidermatum* was more aggressive while *P. ultimum* was more virulent below 20℃. When both *P. aphanidermatum* and *P. ultimum* were mixed as inoculum, they caused plant height reduction at all test temperatures in the range of 12-32℃. Based on reisolations, *P. aphanidermatum* was obtained from about 90% of inoculated seedlings incubated at 24℃ or higher temperatures and *P. ultimum* obtained from 83% of inoculated plants incubated at 20℃ or lower (Table 4).

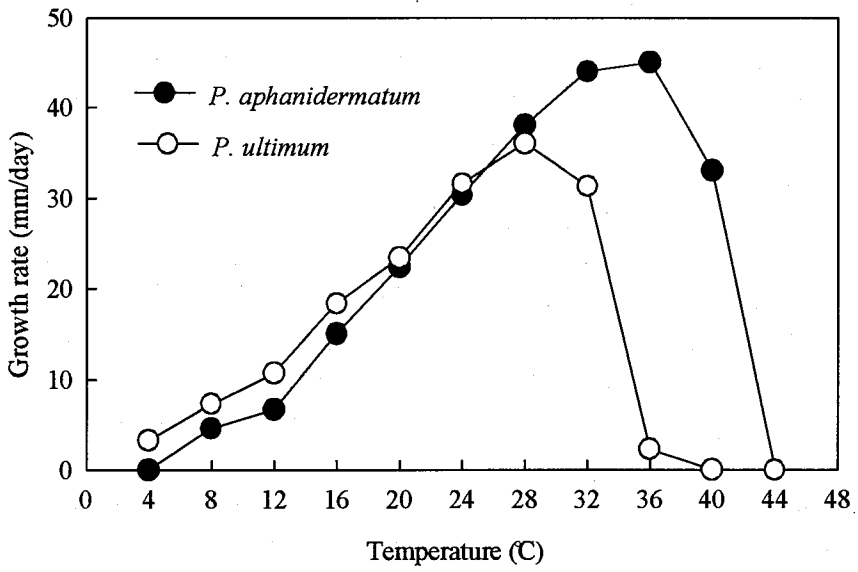

Fig. 3. Effect of temperature on the mycelial growth of *Pythium aphanidermatum* and *P. ultimum* isolated from pea seedlings on patato carrot agar.

Table 4. Effect of temperature on the infection of pea seedlings by *Pythium* spp.

Days after seeding	Pathogen[1]	Stem length (mm) / % of reisolation rate (P.a., P.u.) Temperature (℃)					
		12	16	20	24	28	32
8	CK	13a.[2]/(0, 0)[3]	40b /(0, 0)	98a /(0, 0)	91a /(0, 0)	65a /(0, 0)	39a /(0, 0)
	P.a	12a /(0, 0)	51a /(30, 0)	87b /(46, 0)	63b /(100, 0)	27c /(100, 0)	8c /(93, 0)
	P.u.	12a /(0, 50)	40b /(0, 90)	73c /(0, 100)	64b /(0, 96)	44b /(0, 93)	37a /(0, 53)
	P.a. + P.u.	11a /(0, 50)	33c /(0, 100)	74c /(23, 83)	68b /(93, 23)	43b /(100, 0)	22b /(90,0)
11	CK	27a /(0, 0)	93a /(0, 0)	-[4]	-	×[5]	×
	P. a.	23a /(3, 0)	88a /(50, 0)	-	-	×	×
	P. u.	16b /(0, 100)	56b /(0, 96)	-	-	×	×
	P.a. + P.u.	17b /(0, 96)	53c /(0, 100)	-	-	×	×
17	CK	88a /(0, 0)	-	-	-	×	×
	P. a.	89a /(16, 0)	-	-	-	×	×
	P. u.	24b /(0, 100)	-	-	-	×	×
	P.a. + P.u.	17b /(3, 96)	-	-	-	×	×

[1] CK=Uninfested substrate; P.a.=*Pythium aphanidermatum*; P.u.=*P. ultimum*.
[2] Values followed by the same letter in each column are not significantly different at p=0.05, according to the Duncan's Multiple Range Test.
[3] Numbers in the parentheses indicate the isolation rates of P.a. and P.u. (from left to right).
[4] Pea seedlings had been harvested already.
[5] Pea seedlings ceased to grow.

The experiments were carried out at 16-28 ℃ in growth chambers to study the infection of pea roots by *P. aphanidermatum* and *P. ultimum*. Results indicated that both *P. aphanidermatum* and *P. ultimum* were able to colonize pea roots 3 days after seeding in the infested substrates. The percentage of reisolation for these two fungi were 80-100 and 33-63%, respectively. When the seedlings were first inoculated with *P. aphanidermatum* for 3 days, then transplanted into the substrate infested with *P. ultimum* for 7 days of incubation, they were mainly attacked by *P. aphanidermatum* at the test temperatures of 16-28℃. However, 20% of root samples isolated were *P. ultimum* at 16℃. If seedlings were firstly inoculated with *P. ultimum*, then transplanted to the *P. aphanidermatum* infested substrate, the roots were occupied by both *P. ultimum* and *P. aphanidermatum*, except at 16℃ at which *P. aphanidermatum* was not isolated. The isolation rates of *P. aphanidermatum* were 67-93% at 20-28℃ (Table 5).

DISCUSSION

Commercial production of vegetable pea seedlings in plastic sheet house employing a soilless cultural system was first developed in Taiwan since 1986[3, 4]. However, a previously undignosed root rot soon occurred to threaten the industy to an extent that many farms have been either temporarily or permenently abandoned. *P. aphanidermatum* and *P. ultimum* were found to be the pathogens responsible for the devastating disease (Table 1,2,3).

Table 5. Isolation rates of pathogens from pea roots by cross inoculations with *Pythium aphanidermatum* (P.a.) or *P. ultimum* (P.u.)

Days after seeding	Treatment[1]	% of isolation rate (P.a., P.u.) Temperature (℃)			
		16	20	24	28
3	A	(80, 0)[2]	(90, 0)	(100, 0)	(100, 0)
	B	(0, 63)	(0, 53)	(0, 50)	(0, 33)
	C	-[3]	-	-	-
	D	-	-	-	-
10	A	(100, 20)	(100, 0)	(100, 0)	(100, 0)
	B	(0, 100)	(67, 93)	(100, 73)	(93, 40)
	C	(100, 0)	(100, 0)	(100, 0)	(100, 0)
	D	(0, 100)	(0, 100)	(0, 100)	(0, 100)

[1] Pea seeds were sowed on saw dust pre-infested with *P. aphanidermatum*. Three days after seeding, they were transplanted onto saw dust which had been infested with *P. ultimum* and allowed to grow for another 7 days (A); or otherwise, seeds were sowed on P.u. infested substrate first and then transplanted onto substrate infested with P.a (B); and seeds grown on P.a. (C) or P.u. (D) infested substrate for 10 days to serve as controls.

[2] Numbers in parentheses indicate the isolation rates of P.a. and P.u. (from left to right).

[3] Not tested.

The seasonal occurrence of different *Pythium* spp. resulting in sequential damage to root system has been found in many crops[1, 2, 5, 7, 9]. For instance, cucumber is infected by *P. aphanidermatum* in summer and *P. spinosum* in winter[7]. Hydroponic vegetables were mostly infected by *P. aphanidermatum*, *P. myriotyrlum* and *Pythium* group 'G' in summer, and by *P. dissotocum*, *P. coloratum* and *Pythium* group 'F' in winter[2, 5]. In our study, the occurrence of *P. aphanidermatum* in summer and *P. ultimum* in winter was demonstrated for pea seedlings in soilless culture system, with evidence indicating that temperature was the most important factor in causing this seasonal fluctuation (Fig. 2).

Pythium aphanidermatum is considered an important fungus that caused plant diseases under warm conditions. It damaged the hydroponic vegetables at the temperatures above 23℃ [2], and caused several severe diseases of crops in field soil above 27℃[1, 6, 14]. By contrast, *P. ultimum* was active in cool to moderately warm environments, often causing disease at 15-20℃ [8, 10, 11, 13]. However, the optimum temperatures for mycelial growth on agar medium of *P. aphanidermatum* and *P. ultimum* isolated from pea seedings were 36 and 28℃, respectively, when tested on agar medium (Fig. 3). They infected the pea roots at 16-32℃ (showing symptoms at 20-32℃), and 12-32℃ (showing symptoms at 12-28℃), respectively, in the growth chamber inoculation tests (Table 4). In other words, both pathogens have the ability to infect pea seedlings and cause root rot both in summer as well as winter in the commercial farms, if they are used as inoculum alone in the soilless cultural system. When *P. aphanidermatum* and *P. ultimum* were both present, *P. aphanidermatum* was the primary pathogen at temperatures higher than 24℃,

whereas *P. ultimum* was the primary or sole pathogen at temperatures lower than 20℃ (Table 4; Fig. 2).

Pythium aphanidermatum could not cause severe root rot of sugar beets in fields at temperatures lower than 27℃ [14]. This was ascribed to the slow germination of oospores at low temperatures as influenced by the rhizospheric soil bacteria[12]. The reason for the failure of *P. ultimum* to cause severe diseases of crops at temperatures higher than 20℃ in soil environments[8,10,11,13] has not been determined. Therefore, it is interesting to know how *P. aphanidermatum* and *P. ultimum* attack the root tissues of pea seedlings that had already been infected with the other species, i. e. *P. ultimum* or *P. aphanidermatum* in our study. The experiments on the infection of pea roots by *Pythium* spp. conducted in soilless cultural system in growth chamber at 16-28℃ gave evidnce that *P. aphanidermatum* was a more aggressive root-tissue colonizer at temperature higher than 24℃ and *P. ultimum* was more aggressive at temperatures lower then 20℃ (Table 5). In other words, the root tissues first infected with *P. ultimum* would be infected later by *P. aphanidermatum* at the test temperatures of 20℃ or higher. One of the explainations would be that the inability of *P. ultimum* to cause severe diseases at high temperatures might be due to the competition of *P. aphanidermatum* for the root tissues.

This disease was not effectively controlled by spraying chemicals in numerous attempts made by growers. The avenue of introduction of *P. aphanidermatum* and *P. ultimum* into the soilless cultural system and the means of their transmission will be presented in next paper.

ACKNOWLEDGEMENTS

This study was supported in part by the National Science Council, R. O. C. under ther grant NSC-81-0409-B-005-522. Thanks are given to Dr. R. J. Chiu, Agricultural Biotechnology Laboratoies, National Chung Hsing University for reviewing the manuscript. Part of the first author's master thesis submitted to National Chung Hsing University

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摘 要

黃晉興[1]、**林益昇**[2] 1998 **腐霉菌引起設施栽培豌豆苗根腐病** 植保會刊 40: 397-408 ([1] 台中縣霧峰鄉 臺灣省農業試驗所；[2] 台中市 國立中興大學植物病理學系)

在無土栽培的豌豆苗發生一種前未診斷之根腐病，罹病株嚴重根腐、矮化與死亡，經鑑定是由 *Pythium aphanidermatum* 和 *P. ultimum* 所引起。接種試驗顯示 *P. aphanidermatum* 和 *P. ultimum* 最適感染植株的溫度分別爲 20－32℃及 12－28℃。然而這兩種病原菌共同存在於栽培農場或混合一起在生長箱的接種試驗時，*P. aphanidermatum* 在氣溫高於 24℃的夏季中是主要的病原菌，而 *P. ultimum* 則在氣溫低於 20℃的冬季主導本病害。

(關鍵詞：豌豆苗、根腐病、腐霉菌)